The placenta as modulator of fetal prosperity
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Introduction Part 2: The Placenta as the Basis of Gestational and Fetal Disease

Gestational Hypertensive Disorders

There is ample clinical evidence\textsuperscript{127} that trophoblast cells are able to induce gestational hypertensive disorders as preeclampsia (PE), eclampsia, pregnancy-induced hypertension and Hemolysis, Elevated Liver enzymes and Low Platelet (HELLP) – Syndrome (defined as platelet count < 100 x 10\textsuperscript{9} /l, aspartate aminotransferase ≥ 70 U/l and/or lactate dehydrogenase ≥ 600 U/l).

Gestational hypertensive disorders are highly associated with fetal growth restriction, that in turn is the resultant of placental dysfunction.\textsuperscript{21;128;129} The exact molecular basis is unknown.

Preeclampsia

Preeclampsia is a syndrome with a highly variable expression typically occurring in the second half of pregnancy and is observed in approximately two to three percent of pregnancies.\textsuperscript{130;131} It is a major cause of maternal\textsuperscript{132} and fetal\textsuperscript{133} morbidity and mortality, especially in the 25 percent of cases when the disease is severe, and when it occurs in pregnancies less than 34 weeks gestation. The syndrome is defined as new onset hypertension and proteinuria in a previously normotensive woman. According to the statement of the International Society for the Study of Hypertension in Pregnancy (ISSHP), the operational definition of preeclampsia is a diastolic blood pressure ≥ 110 mmHg on any occasion or a diastolic blood pressure ≥90 mmHg on two separate occasions at least four hours apart in combination with proteinuria ≥ 0.3 g/24 hr., that develops after 20 weeks gestation.\textsuperscript{134} Other international organisations use more or less similar definitions.\textsuperscript{13}

There are multiple predisposing factors for preeclampsia. Although preeclampsia is defined as pregnancy-induced hypertension, pre-existing hypertension is a major predisposing factor to develop superimposed preeclampsia. Primiparity, black race, obesity,\textsuperscript{137} diabetes,\textsuperscript{138} insulin resistance,\textsuperscript{139} pre-existing vascular diseases such as systemic lupus erythematosus,\textsuperscript{140;141} pre-existing thrombophilic disorders as protein S deficiency, activated protein C resistance, hyperhomocysteinemia or anticardiolipin antibodies\textsuperscript{142} are other weakly predisposing factors. There are data reporting an incidence of 50 percent pre-existing renal disease in preeclampsia patients.\textsuperscript{143} The predictive value of all of these factors is not clearly established.\textsuperscript{131} The clinical signs alerting the obstetrician that preeclampsia might develop is the gradual increase of blood pressure, although there are several biochemical
abnormalities (such as haemoconcentration, hyperuricemia\textsuperscript{144}) that precede the clinical disease for weeks. Proteinuria is a late sign and generalised oedema usually becomes apparent in the latter part of the third trimester and progresses until and after delivery. Additionally, signs of severe disease include central nervous system manifestations such as headaches, blurred vision, scotomata and, rarely, cortical blindness. Right upper quadrant or epigastric pain is indicative of liver involvement.

The preeclamptic spectrum varies from very mild to life-threatening with first symptoms presenting very early, in the latter half of the second trimester, to very late during delivery or even in the early postpartum period. In order to define clinically relevant forms of preeclampsia, Ness and Roberts\textsuperscript{145} and von Dadelszen\textsuperscript{146} proposed a classification describing two forms in this clinical spectrum: early- and late-onset preeclampsia.

Both for the early- and late-onset forms of preeclampsia the presence of placental tissue is a prerequisite for the disease.\textsuperscript{127} A fetus is not required, which is illustrated by the fact that hypertension and proteinuria occur with a high incidence in hydatiform mole.\textsuperscript{147} Placental localisation in the uterus is also not required since preeclampsia can develop during an abdominal pregnancy. A case report describes the persistence of preeclampsia until removal of the placenta, 99 days after delivery of the fetus from an abdominal pregnancy.\textsuperscript{148}

Clinically, the most prominent difference between the early-onset and late-onset class is the presence or absence of fetal growth restriction. In a nationwide study in Norway, including over 670,000 births, neonates born after early-onset preeclampsia were lighter, shorter and leaner than neonates from either late-onset preeclampsia or normotensive pregnancies.\textsuperscript{149} Early-onset preeclampsia is seen as the consequence of poor placentation that is causal to the impairment of placental and fetal growth.\textsuperscript{145;146} It is generally recognized that early-onset preeclampsia has most serious consequences for neonatal morbidity\textsuperscript{150} and is accompanied by high recurrence rates.\textsuperscript{151;152} Furthermore, early-onset preeclampsia is associated with maternal dislipidemia.\textsuperscript{153}

In late-onset preeclampsia, placental dysfunction is not pronounced as in early-onset preeclampsia. Clinical symptoms in late-onset preeclampsia are modulated by generalized maternal endothelial dysfunction that is triggered by pre-existing maternal endothelial disease\textsuperscript{145;146;154} such as diabetes, renal disease, obesity. This predisposition has been termed the metabolic syndrome of pregnancy.\textsuperscript{155;156}
Pathogenesis
There are several processes essential to the pathogenesis of preeclampsia: impaired trophoblast invasion leading to defective placentation, placental oxidative stress and systemic endothelial activation. Figure 7 summarizes them in the simplified form of a cascade. As can be seen in the figure, the processes involved in the pathogenesis are local as well as systemic. Various steps in this cascade have joint effects on others, and in combination with either pre-existent maternal endothelial dysfunction or imbalance in clotting factors they lead to the complex spectrum of gestational hypertensive disorders, ranging from late-onset preeclampsia to early-onset preeclampsia and HELLP syndrome.

Figure 7: Pathogenesis of Preeclampsia.
Summary of the processes that attribute to the syndrome of early- and late-onset preeclampsia, as well as HELLP. Figure modified from Redman.157

Defective trophoblast invasion and abnormal placentation
Immune maladaptation is currently the leading theory with respect to abnormal placentation in preeclampsia. The importance of immune factors
is demonstrated by the presence of antibodies against endothelial cells and immune complexes in uterine spiral arteries, kidneys, liver and skin of preeclamptic women. The placenta is in part an allograft. Fetal trophoblast cells are in very close contact with blood from the genetically different maternal system, inducing local immunological factors. As described on page 14 (Placental development, Implantation and Invasion) cytotrophoblast invasion into endometrium, myometrium and spiral-shaped maternal blood vessels is essential to normal placentation. The maternal lymphocytes encountered by the trophoblast cells invading the placental bed are mainly natural killer cells. They recognize the unique HLA combination displayed by the invading trophoblast cells, thereby permitting deep invasion into an immunologically different system. In preeclampsia this invasion is abnormally shallow and fails to modify the maternal spiral arteries. Microscopically, the preeclamptic placenta indeed shows loss and distortion of villi, focal syncytial necrosis, decreased syncytial activity, cytotrophoblast hyperplasia as well as degeneration of cytotrophoblast cells and the presence of small fetal capillaries with bulbous endothelial cells. The general concept, that prior exposure to allografts protects against a rejection reaction also holds for preeclampsia as the risk decreases in subsequent pregnancies, whether the initial pregnancy was carried to term or ended in abortion. Blood transfusion and increased exposure to semen (e.g. length of cohabitation, use of oral contraceptives) are also associated with a decreased risk of preeclampsia. On the other hand, artificial donor insemination and oocyte donation both lead to an increased risk.

**Placental ischemia resulting in oxidative stress**

The relatively high resistance in abnormally developed spiral arteries results in placental ischemia at the end of the first trimester of pregnancy. Ischemia causes the formation of reactive oxygen species which are normally offset by the activity of local anti-oxidants as glutathione S-transferase. Preeclamptic women can have increased amounts of circulating fatty acids that in turn are a target for reactive oxygen species, initiating a self-propagating chain reaction. Based on this biological principle and the hypothesis that preeclamptic women might suffer from a deficient anti-oxidant system, clinical trials evaluating the effect of anti-oxidant therapy (vitamin C and vitamin E) have been performed. Although antioxidant therapy is able to change biochemical parameters in women at risk of preeclampsia, it is unable to decrease the incidence of preeclampsia and has unexpected negative effects with respect to fetal growth.

**Systemic endothelial dysfunction**

Oxygen radicals generated in the placenta are currently seen as the
main causal agents to the generalized endothelial damage typical of preeclampsia. Renal endothelial damage results in proteinuria, liver cell damage is reflected by the elevation of aminotransferase enzymes and vascular constriction and oedema in the brain results in headaches and visual disturbances, with eclampsia and cerebral haemorrhage as very severe consequences. The resulting imbalance of endothelium-derived vasodilators prostacyclin and nitric oxide with vasoconstrictor factors further aggravate peripheral vasoconstriction and hypertension. Additional imbalance in clotting factors can lead to disseminated intravascular coagulopathy, clinically known as HELLP syndrome. Table 2 on page 30 describes some of the mediators of generalized endothelial activation. Strikingly, all the factors that predispose for preeclampsia are also risk factors for endothelial diseases, atherosclerosis in particular, and it has been reported that women with a preeclamptic pregnancy have an increased risk of cardiovascular disease in later life. More insight into the pathogenesis of preeclampsia will therefore help understanding vascular disease later in life.

The molecular basis of preeclampsia

In order to identify genes causal to the development of preeclampsia or HELLP syndrome, quite a number of polymorphisms and mutations in relevant genes have been investigated. Table 2 summarizes genetic analyses published in relation to gestational hypertensive disorders between 1989 and 2007 categorised according to their putative role in the pathogenesis of preeclampsia. Even though involvement of the cascade of processes discussed in the previous paragraphs is substantiated, no strong single causal factor in any of these processes has been identified. A population-based Swedish cohort study on the recurrence of preeclampsia in second pregnancies demonstrated that over 50% of the preeclamptic phenotype is due to genetic factors. This observation is in contrast with the lack of concordance in the occurrence of preeclampsia between pregnant monozygotic twins. While the majority of cases of preeclampsia and HELLP syndrome are not familial, the collection of rare familial cases in a number of geographic areas has enabled linkage studies. These studies identified chromosomal regions on 2p12, 2p13, 2p25, 4q, 7q33, 9p13, and 10q22 as susceptibility loci. Strikingly, there is little concordance between studies and analysis of candidate genes in these regions has not been very successful in identifying causal genes. One exception is an inactivating mutation of the long chain 3 hydroxacyl coA dehydrogenase (LCHAD) that causes HELLP syndrome when a homozygous fetus is present. These mutations are not frequent in pregnancies complicated by HELLP syndrome.
and can explain only a very small proportion of cases.\textsuperscript{194} The maternal inheritance of a variant of the STOX1 gene, common within the population, has been reported as causal to Dutch familial preeclampsia.\textsuperscript{195}

<table>
<thead>
<tr>
<th>Process</th>
<th>Genes Considered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune adaptation</td>
<td>HLA-DR, HLA-DQB1, HLA-DPB1, HLA-G, IGF2, IGF2R, IgG-CRHC, IL1, MMP1, TNFa</td>
</tr>
<tr>
<td>Placentation and Angiogenesis</td>
<td>ADAM12, ERVWE1, FOS, GCM1, INHB, IGFBP3, ITGAI, ITGB, JUN, MBL2, MMP7, MMP9, MMP10, MMP13, MMP15, NKB, sENG, sFlt, STOX1, TGFb, VEGF</td>
</tr>
<tr>
<td>Placental ischemia and oxidative stress</td>
<td>CASP10, CAT, EPHX1, GSH-Px, GSTP1, HIF1, LDHAL4, LDHB, LPL, LPLR, MTHFR, SOD, TNFRSF25, VLDLR</td>
</tr>
<tr>
<td>Endothelial activation and cytokine production</td>
<td>ALOX5, APOC3, apoE, CD14, CEACAM8, COX2, CTLA4, EDN, FSTL3, GNB3, IFNG, IL\textsubscript{10/b}, IL\textsubscript{6}, IL\textsubscript{10}, LIPC, LIPE, LPL, LTA, NFkB, NO, NPY, TNFa, TNFaR</td>
</tr>
<tr>
<td>Vasodilator and vasoconstriction factors</td>
<td>ACE, AGT, AGTR1a/b, AGTR1b, ANP, APLN, BDKRB1, CAPN10, CALCRL, DDAH1, EDN1, ecNOS, HPGD, KLK1, NOS2A PCS, PTGS2, pro-ANF, PTHR, RAMP1, REN, SCNN1, TBX</td>
</tr>
<tr>
<td>Clotting factors</td>
<td>APC, F2, F7, F11, FVL, PAI1, PAR1, Plasminogen, THBD</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>ADIPOQ, COX1, LCHAD, LEP, LEPR, LIPC, HSD11B2, LIPC, PPARG, VDR</td>
</tr>
</tbody>
</table>

Table 2: Genes that have been reported in relation to preeclampsia. Extracted from the Pub Med search Gene\[title/abstract\] AND Preeclampsia\[MeSH\] between 1989 and March 2008.
Strategies to Elucidate the Molecular Basis of Preeclampsia

Impediments to preeclampsia research
A main impediment to preeclampsia research is the lack of placenta material from the first trimester, the time the basis of the disease is established. Changes in placentation, and the cascade of oxidative- and inflammatory damage most likely take place early during gestation and not by the time of delivery, when placental bed biopsies are usually taken. As preeclampsia is a very heterogeneous disease, it is extremely important that the criteria on which subjects are recruited in clinical studies are clearly defined and strictly applied. If this is not the case, the non-uniformly defined patient populations limit the interpretation and association of findings. Characterization of the disease phenotype should be done preferably according to the ISSHP criteria and the diagnosis should be made only after delivery. As can be seen in Table 3 overleaf, not all studies fully meet generally accepted disease criteria. The complex inheritance pattern of susceptibility for preeclampsia and the interactions with environmental factors demonstrate that, with rare exceptions, preeclampsia is not a monogenic disease. Illustrative of the complexity of genetic research is the large number of contradictory reports on the contribution of individual genes to the pathogenesis of preeclampsia. The fact that different studies compare different determinants prohibits generalization of studies. To exemplify, studies on endothelial nitric oxide synthase (ecNOS), published in relation to preeclampsia are summarized in Table 3. The study populations are heterogeneous, varying from gestational hypertension or moderate late-onset to severe early-onset preeclampsia to cases with placental abruption. This, in combination with use of either fetal (placental) or maternal material investigating either DNA, RNA or protein, makes it difficult to generalize the findings. When these studies are pooled and evaluated, there is no increased risk of preeclampsia, neither under a recessive nor a dominant genetic model, as has been shown in a recent meta-analysis.

Animal models
Although there are several animal (sheep, monkeys and rodents) models, none properly reflects the pathophysiological mechanisms of human preeclampsia. The oldest model in sheep is a pregnancy-induced hypertension model, provoked by a 4-day fasting period during early gestation. Apart from hypertension, this condition bears little resemblance to the human preeclampsia syndrome. Moreover, both placental morphology and the placentation process of epitheliochorial placentas, (see Figure 4 page 13) in sheep are quite distinct from human. Arterial occlusion in rhesus monkeys, which have discoid placentas of hemochorial structure just like humans,

<table>
<thead>
<tr>
<th>Study</th>
<th>Study Population</th>
<th>Clinical PE Criteria</th>
<th>Material</th>
<th>Biomarker</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasiell 1998[198] Sweden</td>
<td>13 nonsevere PE, 7 PE and SGA, 8 SGA, 41 control pregnancies</td>
<td>ISSHP</td>
<td>Placental tissue</td>
<td>RNA level of eNOS / Total nucleic acids</td>
<td>ecNOS level significantly higher in complicated- than in normal pregnancies</td>
</tr>
<tr>
<td>Yoshimura 2000[199] Japan</td>
<td>80 severe PE, 35 nonsevere PE, 37 superimposed and 170 pregnant control women</td>
<td>NHBPEP</td>
<td>Maternal DNA</td>
<td>Glu298Asp ecNOS variant</td>
<td>T allele more frequent in subjects with severe PE than in either nonsevere PE or control</td>
</tr>
<tr>
<td>Faxen 2001[200] Sweden</td>
<td>8 nonsevere PE and 12 from normal pregnancies</td>
<td>ISSHP</td>
<td>Placental tissue</td>
<td>mRNA level of ecNOS / RNA GAPDH</td>
<td>mRNA expression was significantly higher in both myometrium and placenta</td>
</tr>
<tr>
<td>Tempfer 2001[201] Germany</td>
<td>24 severe PE and 24 nonpregnant women, with no history of hypertension</td>
<td>ISSHP</td>
<td>Maternal DNA</td>
<td>Glu298Asp ecNOS variant</td>
<td>No association</td>
</tr>
<tr>
<td>Orange 2003[202] Australia</td>
<td>14 nonsevere PE and 4 gestational hypertension, 12 pregnant control women</td>
<td>ASSHP</td>
<td>Placental tissue</td>
<td>Immunohistochemistry, antibody specific for eNOS</td>
<td>No significant difference in eNOS in either villous or decidual staining intensity</td>
</tr>
<tr>
<td>Hakli 2003[203] Finland</td>
<td>132 nonsevere PE, 113 pregnant control women</td>
<td>ISSHP</td>
<td>Maternal DNA</td>
<td>Glu298Asp ecNOS variant</td>
<td>No association</td>
</tr>
<tr>
<td>Yoshimura 2003[204] Bangladesh</td>
<td>112 severe PE and 119 control women</td>
<td>NHBPEP</td>
<td>Maternal DNA</td>
<td>Glu298Asp ecNOS variant</td>
<td>No association</td>
</tr>
<tr>
<td>Landau 2004[205] USA</td>
<td>64 non-severe PE, 397 pregnant control women</td>
<td>AmCOG</td>
<td>Maternal DNA</td>
<td>Glu298Asp ecNOS variant</td>
<td>No association</td>
</tr>
<tr>
<td>Ohta 2004[206] Japan</td>
<td>131 pregnant women with PE and 327 pregnant control women</td>
<td>ISSHP</td>
<td>Maternal DNA</td>
<td>Glu298Asp ecNOS variant</td>
<td>No association</td>
</tr>
<tr>
<td>Serrano 2004[207] Colombia</td>
<td>322 PE and 522 control women</td>
<td>NHBPEP</td>
<td>Maternal DNA</td>
<td>Glu298Asp ecNOS variant</td>
<td>TT variant more frequent in PE</td>
</tr>
<tr>
<td>Wang 2004[208] Australia</td>
<td>15 severe PE, 5 nonsevere PE, 5 normotensive controls</td>
<td>ASSHP</td>
<td>RNA samples from HUVEC cells</td>
<td>RNA level of eNOS / RNA actin</td>
<td>Decreased RNA level in PE samples</td>
</tr>
<tr>
<td>Hillerman 2005[209] South Africa</td>
<td>50 severe PE and 50 abruptio placentae and 50 pregnant control women</td>
<td>NHBPEP</td>
<td>Maternal DNA</td>
<td>Glu298Asp ecNOS variant</td>
<td>T allele more frequent in the abruptio group than control group and PE and abruptio compared to PE alone</td>
</tr>
</tbody>
</table>
induces placental dysfunction with hypertension and fetal growth restriction as a consequence. This model mimics placental ischemia and its consequences, but does not affect renal function.

There are several rodent models in which the placentation process is influenced by reduced uterine perfusion or the administration of endogenous agents. Endotoxin, desoxycorticosterone acetate, nitric oxide synthase inhibitor and the angiogenesis inhibitors TNP470 or Suramin each produce a variety of preeclampsia-like symptoms and fetal growth restriction. Pregnancy-associated hypertension and fetal growth restriction are present in spontaneous hypertensive and heart failure (SHHF) rats, who have chronic hypertension, insulin resistance, and renal impairment as they age. Rats injected with sFLT and sENG develop hypertension and proteinuria or even HELLP syndrome. Transgenic models also exist: p52Kip2 mice have hypertension, proteinuria and coagulation disorders, whereas pregnant BHP5 mice have hypertension, late-gestational proteinuria and progressive renal glomerulosclerosis with growth restriction in the litter. Although rodents have a hemochorial discoid placental structure resembling human, there are several differences between humans and rodents. In human, rat and mouse gestation takes respectively 280, 22 and 20 days while implantation takes place respectively at day 6, 5 and 4. Therefore, in human preeclamptic pregnancy, placenta and fetus are faced with progression of the disease for the major part of gestation, while in rodents this time span is relatively short.

Cell culture and tissue culture
Since the placenta becomes available after delivery, it is then accessible for gene expression studies and primary cell culture. Trophoblast- or endothelial cell cultures are ideally suited to investigate physiological pathways under controlled conditions. As an example, this type of experiment demonstrated secretion of TNFα from hypoxic trophoblast cells mediating the inflammatory response. Cell culture can never be the equivalent for the in vivo situation. The fact that the cellular composition of the placenta changes dramatically during gestation, makes this more true for placenta.

Studies on gene expression
Every cell in the human body has the same genetic repertoire, consisting of genes located on chromosomes. The protein-coding sequences (exon sequences) that are usually dispersed over a gene are divided by intervening sequences (intron sequences). During RNA splicing the exon sequences are fused, rendering mature mRNA molecules of which the open reading frame is translated into protein. (Figure 8, overleaf)
Basal common cellular processes occur in all cell types and are dependent on proteins encoded by housekeeping genes. As for all tissues and organs, placental tissue-specific gene expression defines the proteins that form the basis for its growth, development and function. Depending on the number of samples in which the transcription level has to be determined and the total number of genes that need to be investigated, several molecular biology techniques are available. Northern blot analysis requires relatively large amounts of RNA and is suitable to analyse the semi quantitative expression level of a few genes. The expression level of a specific single gene can be assessed by the far less material- and time-consuming semi-quantitative polymerase chain reaction (PCR), or real-time PCR techniques. The development of high-throughput biotechnology techniques such as microarray analysis and serial analysis of gene expression (SAGE), in combination with major advances in bioinformatics has opened ways to investigate complex diseases at the RNA level.

A microarray is a solid surface to which tens of thousands DNA spots, representing genes, are attached. Hybridization of fluorescent labelled RNA derived from individual cell cultures or tissue samples allows the monitoring of the expression level of genes present on the array. This way, an array yields expression signals from tens of thousands of genes over series of individual samples. Arrays can contain previously identified genes but also Expressed Sequence Tags (ESTs), partial cDNA sequences derived by
sequencing clones from cDNA libraries. The main limitation of the microarray technique is that only previously selected transcripts or ESTs are analyzed. Secondly, genes expressed at lower levels or in a small subset of cells are likely to be missed in this multigene technique. Finally, the vast amount of data also cause a very unfavourable signal to noise ratio, hindering the identification of truly important genes.

With respect to placental function, microarray technology has been used in different stages of cultured trophoblast cells, establishing different processes of differentiation. Also, there are models comparing gene expression in decidua and villi in placental samples using microarray technology as well as models of trophoblast invasion and placental hypoxia. Microarray analysis in preeclamptic placenta has suggested up regulation of glycogen phosphorylase as well as subsets of apoptosis- and calcium metabolism-related protein metabolism-related and obesity-related genes. Differential regulation of tumour suppressor and growth regulatory genes are reported to play a role in the pathogenesis of severe early-onset preeclampsia. Recently also genes related to an imbalance of reactive oxygen metabolites, abnormal trophoblast invasion, disorders of lipoprotein metabolism and growth factors have been found specifically differentially regulated in preeclampsia.

Serial Analysis of Gene Expression (SAGE)

The SAGE technique is a tool to generate complete expression profiles at the transcriptional level. The traditional generation of a SAGE library starts with a milligram of mRNA from the tissue or cell type of interest. High-quality double stranded cDNA is synthesized using biotinated oligo-dT, captured and cleaved with the anchoring enzyme NlaIII, yielding 3' cDNA fragments with an average size of 265 bp. After ligation to specific linkers, the cDNA pool is digested with BsmF1 that recognizes a sequence in the linker and cuts a variable number of nucleotides downstream, generating an mRNA-specific SAGE tag. These SAGE tags are ligated to ditags, PCR amplified, concatenated and sequenced. The concatenation of tags in a serial fashion makes SAGE a high-throughput technique for a limited number of samples. The resultant SAGE library consists of a list of ten-to-hundred thousands of 10 base pair SAGE tags that each occur 1 or more times. The quantitative properties are based on simply counting the number of times a tag occurs. The qualitative properties are based on the fact that a 10 base-pair sequence adjacent to the most 3' CATG site of a mRNA molecule contains sufficient information to uniquely identify the corresponding transcript. Since alternative splicing, alternative polyA adenylation and polymorphisms result in multiple tags corresponding to a single gene, tag to gene allocation is actually the most difficult step in SAGE library analysis.
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Figure 9: Schematic representation of the SAGE method.

Typically, each SAGE library also contains tags that can not be linked to a transcript with known or inferred function: the so-called NoMatch tags. They are the leads to identify novel genes and transcripts. The public availability of tens of SAGE libraries of different human tissues enables the recognition of tissue (i.e. placenta-) specific transcripts. SAGE is the only available tool to generate complete yet comprehensive expression profiles without prior availability of transcript information. It provides the opportunity, with disease phenotype-related No Matches as leads, to discover novel disease-specific genes. This concept has been extensively validated for cancer research.235
Aim and scope of this Thesis

With optimal placental function as prerequisite for fetal prosperity, this thesis describes the relation of placental development with maternal gestational and fetal disease. (Chapter 1) The consequences of placental function are investigated by the analysis of placental weight birth weight in Chapter 2 and 3. Since the placenta has an important role in hormone metabolism and hormonal interaction between mother and fetus, we describe thyroid hormone interactions with respect to fetal growth restriction and gestational hypertensive disorders in Chapter 4 and 5. Molecular biological techniques and bioinformatics are used to study gene expression relevant to characterization of PE and HELLP syndrome in Chapter 6 and to develop gestational age specific placental profiles in Chapter 7.
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